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YELLOW-LEAFBLOTCH OF ALFALFA CAUSED BY THE FUNGUS PYRENOPEZIZA MEDICAGINIS[1]

By FRED REUEL JONES

Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the spring of 1914, when the writer began a study of the foliage diseases of alfalfa (*Medicago sativa*) in the vicinity of Madison, Wisconsin, special attention was given to the leafspot caused by the fungus *Pseudopeziza medicaginis*, which is generally regarded as the most important disease of this plant. But it soon became apparent that another disease, which had not previously been mentioned as occurring in the United States, was responsible, under certain conditions at least, for even greater damage to the crop than the well-known leafspot. A study of this disease was undertaken in addition to the work on leafspot, and a brief report was made in 1916 (*10*).[2] Since that time the work on this disease has been continued, stimulated by an increasing recognition of its importance. The results are presented in the following pages.

THE DISEASE

DISTRIBUTION

During the two summers which have elapsed since the yellow-leafblotch was first noted in the United States, there has not been opportunity to make a complete survey of its distribution. It has been collected by the writer or correspondents in Vermont, New Jersey, Virginia,

[1] This paper presents one part of the results of a study of a group of alfalfa and clover diseases, which was begun in the Department of Plant Pathology at the University of Wisconsin in 1914. In 1916 the writer became a collaborator of the Office of Cotton, Truck, and Forage Crop Disease Investigations, of the Bureau of Plant Industry, and through cooperation between the Bureau and the Wisconsin Agricultural Experiment Station, the scope of the work was extended. The work has been done under the immediate direction of Dr. L. R. Jones, to whom grateful acknowledgments are expressed. Other members of the Department of Plant Pathology at the University of Wisconsin have contributed many valuable suggestions, and several correspondents have furnished specimens and information which have added greatly to the value of the work.

[2] Reference is made by number (italic) to "Literature cited," p. 329.

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New York, Ohio, Kentucky, Tennessee, Wisconsin, Minnesota, Iowa, Kansas, South Dakota, California, Idaho, Oregon, and Washington. To judge from the wide range of conditions under which the disease has already been found, there appears to be no reason to doubt that it will be found in practically all of the important well-established alfalfa-growing regions in the country. It is possible that this leafblotch is not yet introduced into some of the newer and more isolated districts.

Outside the United States the only record of its occurrence in America is from Argentina (5). In Europe the disease has been found in Austria, Germany, France, and Italy, where it has long been known, though not regarded as important.

ECONOMIC IMPORTANCE

To what extent the yellow-leafblotch is a serious economic factor in the culture of alfalfa it will be impossible to state with confidence until a larger amount of data has been collected over a period of years; but that it does produce large loss of foliage under certain conditions is beyond doubt. From observations made largely at Madison, Wis., it appears to vary widely in the severity of its attack on different cuttings from the same field, depending largely on the abundance of infectious material and on weather conditions favoring infection. Under favorable conditions it has caused much larger loss of foliage than the leafspot caused by the fungus *Pseudopeziza medicaginis*. For instance, when the first crop was being cut on the experiment station farm at Madison, on June 19, 1916, it was estimated by observers that this disease had destroyed about 25 per cent of the foliage, and that at least 75 per cent of that remaining was more or less infected. In the summer of 1915 the disease was twice reported in private correspondence to the writer as of economic importance, by Dr. M. P. Henderson in the Rogue River Valley, Oreg., and by Mr. L. E. Melchers in Kansas. In a recent report (*11*) Melchers has stated that in many places in Kansas in 1916 the disease caused a loss of 40 per cent of the foliage of the first and second crops.

Frequently only a small part of the diseased foliage falls off and is lost. The fungus does not usually kill the invaded tissue until a large part of the leaf has been penetrated; but invaded tissue easily becomes water-soaked during rains, whereupon the leaf dies, and upon the return of dry weather it quickly shrivels and falls.

DESCRIPTION (PL. D)

Although the disease varies considerably in appearance, it has characteristics which make it easily distinguishable from other alfalfa diseases. The first visible evidence of its presence on the leaf is usually a blotch of characteristic yellow color, with its longer diameter parallel to the

direction of the veins. In a few days the blotch may extend from the midrib to the margin if the leaf is small. In shape it remains somewhat elongate, as though restricted by the veins. It does not appear that an entire leaflet is ever invaded by a single infection. In color it becomes a deeper yellow, often approaching a brilliant orange on the upper surface, and a little paler beneath.

Shortly after the appearance of the yellow blotch, sometimes at its first appearance, the central portion of the area shows on the upper surface of the leaf small orange-colored points which indicate the location of pycnidia. They may be very inconspicuous at first. They may be closely grouped along the center of the blotch if it is small, or may be more scattered if it is large. A smaller number of pycnidia usually emerge later on the lower side of the leaf. They soon become deep brown, or even almost black. On mature foliage which has lost its deep-green color, and especially upon plants with narrow leaflets, these elongate groups of dark-colored pycnidia may be the most easily recognized characteristic of the disease.

The diseased areas may not die and dry out for a long time, especially the foliage may take on a rusty-yellow color with no very distinct lesions, but with pycnidia scattered more or less uniformly over the leaf. Under very dry conditions such leaves may dry out quickly with little change in color. But during protracted rains they quickly become water-soaked and fall off.

Under very favorable conditions of protracted cool weather, especially in the autumn, these diseased areas may show on the lower surface of the leaf a number of small black bodies which may develop into apothecia before the death of the entire leaf. Ordinarily apothecia do not develop until after the death of the entire leaf (Pl. 26). At this time the diseased areas become very dark brown or black in color, in contrast to the light-brown color of that part of the leaf which was not diseased. If the dead leaves remain attached to the plant, they often show a tendency to curl spirally with the lower surface outward. The first apothecia appear as small black dots, rarely as much as a millimeter in diameter, scattered along the diseased areas on the lower surface of the leaf. Later, a few may appear on the upper surface. Occasionally newly infected leaves which have been killed prematurely by frost may develop scattered apothecia which are not located on blackened areas; but if the disease has been at all abundant and the plants have not been cut close too late in the fall, there are usually a sufficient number of dead leaves with characteristic blackened areas present to make possible the recognition of the presence of the disease even during the winter months.

The disease occurs on stems as well as leaves, but not as abundantly. From field observations it appears that the fungus usually requires a longer time to produce a conspicuous lesion on the stem than on the

leaf. Lesions appear as elongate yellow blotches, which soon turn a dark chocolate-brown.

The killed area rarely girdles the entire stem. Pycnidia are not as abundant as on the leaf. Apothecia have been found on stems but once, and then not well developed or abundant. No case has yet been observed where a plant has appeared to be seriously affected by the presence of this disease on the stem.

THE CAUSAL ORGANISM

TAXONOMY

In the previous report (*10*) the writer expressed the opinion that the fungus causing the yellow-leafblotch of alfalfa was *Phyllosticta medicaginis* (Fuckel) Sacc. (originally described as *Ascochyta medicaginis* Fuckel; *4*), and that experimental evidence indicated that this species of Phyllosticta is the conidial stage of *Pyrenopeziza medicaginis* Fuckel, which appears later on the dead leaves of this host. Additional experimental evidence has confirmed this opinion.

Shortly after this report had been made, an earlier name applied to the conidial stage of this fungus was found. A comparison of the descriptions indicated that *Phyllosticta medicaginis* was identical with a fungus which had previously been described by Desmazieres under the name "*Sporonema phacidioides*" (*3*). A comparison of the type specimen of *S. phacidioides* [1] with the type specimen of *Phyllosticta medicaginis* [2] was kindly made by Dr. W. G. Farlow. He decided that the fungi to which the two names were applied were identical, and that the names should therefore be regarded as synonyms.

In addition to the names already mentioned, "*Gloeosporium morianum* Sacc." has been suggested by Von Höhnel (*6*) as belonging to this synonymy. From an examination of the description of *G. morianum* it appeared to the writer that Von Höhnel was probably correct. Therefore specimens of *Sporonema phacidioides* were sent to Dr. P. A. Saccardo with the request that they be compared with the type specimen of *G. morianum*. Dr. Saccardo kindly made the comparison and assured the writer that they are identical. Thus, the synonymy of the conidial stage of the fungus is as follows:

Sporonema phacidioides Desm., 1847, Ann. Sci. Nat. Bot., s. 3, t. 8, p. 172–192.

Ascochyta medicaginis Fuckel, 1869/70, Jahrb. Nassau. Ver. Naturk., Jahrg. 23/24, p. 388.

Phyllosticta medicaginis (Fuckel) Sacc., 1884, Syll. Fung., v. 3, p. 42.

Gloeosporium morianum Sacc., 1892, Syll. Fung., v. 10, p. 458.

[1] Desmazieres, J. B. H. J. PLANTES CRYPTOGAMES DE FRANCE. fasc. 33, no. 1645. Lille, 1847.

[2] Fuckel, Leopold. FUNGI RHENANI EXSICCATI. no. 488. 1863.

The first description of the ascigerous stage of *Sporonema phacidioides* was given by Fuckel in 1870 (*4*) under the name "*Pyrenopeziza medicaginis*." No record of any other collection of this fungus than Fuckel's type collection[1] has been found. Since *Pyrenopeziza medicaginis* Fuckel is the name of the ascigerous stage, it is the name which should be applied to the fungus as a whole, according to present rules of nomenclature.

The fact that *Sporonema phacidioides* is found to be the conidial stage of *Pyrenopeziza medicaginis* is a matter of considerable interest, because this fungus has long been regarded as a possible conidial stage of the leafspot fungus *Pseudopeziza medicaginis*. This misconception appears to have developed in the following manner. When, in 1847, Desmazieres described *S. phacidioides*, making it the type species of the new genus (*3*), he noted that the pycnidia opened by irregular valves in much the same manner as the apothecia of *Pseudopeziza medicaginis* (then known as *Phacidium medicaginis*), and that it occurred in association with this species of Phacidium. This common morphological feature and the association of the fungi suggested the species name "*phacidioides*." Next, in 1865, during the course of a discussion of *Pseudopeziza medicaginis*, Tulasne (*14*) made a note of the fact that the two fungi occur together. Although from a careful study of Tulasne's statement it does not appear that he intended to indicate more than an incidental association of the two fungi, yet subsequent writers generally have interpreted it as pointing out a probable relationship. For instance, Brefeld (*1*) says:

> Von ihr [*Pseudopeziza medicaginis*] giebt Tulasne Pycnidien mit kleinem länglich eiformigen Sporen an, die in ahnlicher Weise wie die Ascusfruchte ihre Hymenium basslegen sollen.

Thus, scattered through the literature of *Pseudopeziza medicaginis* from the time of Tulasne's statement, are found, chiefly in European literature, a large number of references to the conidial stage of this fungus which clearly indicate that *Sporonema phacidioides* was referred to, although it was not always specified by name. The persistence of the idea that *S. phacidioides* is the conidial stage of *Pseudopeziza medicaginis* is all the more surprising, in view of the fact that there appear to have been no recent collections of the former on alfalfa, and all the older collections were from France and Belgium only.

The reason for the scarcity of collections of *Sporonema phacidioides* is obviously as follows: In 1870 Fuckel (*4*) had described what now appears to be the same fungus under the name "*Ascochyta medicaginis*." In 1884 Saccardo (*13*) transferred the species to the genus Phyllosticta. Thus, almost all the collections of this fungus outside of France and Belgium were placed under one or the other of these two names. And

[1] Fuckel, Leopold. FUNGI RHENANI EXSICCATI. no. 1594. 1865.

curiously enough, although the fungus was widely known under these names, no one appears to have suggested a possible relationship of the fungus under this name with *Pseudopeziza medicaginis* as had been suggested for the same fungus under the name "*Sporonema phacidioides*."

Under the name "*Gloeosporium morianum*," by which it was described by Saccardo in 1886 (*12*), the fungus does not appear to have become widely known. Only a single subsequent reference to an identification of the fungus by that name (*2*) has been found.

Thus far the writer has traced the development of the misconception of *Sporonema phacidioides* as the conidial stage of *Pseudopeziza medicaginis* on alfalfa. This misconception appears to have gone even farther, and to have been responsible for three references to Sporonema on clover (*Trifolium pratense*) as the conidial stage of *Pseudopeziza trifolii*. The first of these is in a note over the signature of G. Von Niessl appended to No. 2057 of Rabenhorst's Fungi Europaei.[1] This collection is designated "*Pseudopeziza trifolii* (Bernh.) Fckl. St. *conidiophorus*." At the end of the note describing this fungus occurs this sentence:

> Das ganze Gebilde entspricht der alten Gattung Sporonema.

The other references to species of Sporonema on clover are by Jaczewski (*7*, *8*, *9*). In the second reference *Sporonema phacidioides* is specifically stated as occurring in Russia as the conidial stage of *Pseudopeziza trifolii*.

Since field observation and inoculation experiments both indicate that Sporonema does not occur on clover, it is a matter of interest to know what fungus on this host has been called by that name. Through the kindness of Prof. Thaxter, the writer has been permitted to study a portion of Von Niessl's collection in the Harvard herbarium. The fungus found here appears to be identical with *Gloeosporium trifolii* Pk. Owing to war conditions, it has not been possible to obtain specimens of the fungus which Jaczewski regards as the conidial stage of *Pseudopeziza trifolii*. For the present the writer can only assume tentatively that it is the same fungus that is given under that designation by Von Niessl. Since the general character of *Gloeosporium trifolii* is not greatly different from that of *Sporonema phacidioides*, it is not difficult to see why Von Niessl and Jaczewski, holding *Sporonema phacidioides* as the conidial stage of *Pseudopeziza medicaginis* on alfalfa, should have come to regard this species of Gloeosporium as the corresponding conidial stage of *Pseudopeziza trifolii* on clover.

MORPHOLOGY

MYCELIUM.—The mycelium within the leaf tissue varies greatly in diameter. Many of the lateral branches are very small, while older hyphæ may become greatly swollen. Hyphæ are found largely within, but

[1] Rabenhorst, G. L. FUNGI EUROPAEI EXSICCATI. new ed., cent. 21, no. 2057. Dresdae, 1876.

sometimes between the cells. When the tissue is thoroughly invaded, the interior of the larger palisade cells and many other large parenchyma cells become lined with a thick-walled cellular fungus layer. Finally the leaf tissue almost disappears and becomes replaced with a fungus stroma.

In culture the mycelium develops largely in the substratum, where it forms a dense mat that does not appear to amalgamate into a stroma, though in old cultures it may produce a stroma at the surface of the culture medium. The aerial mycelium usually appears as a close felt of hyphæ arising from the submerged mycelium. In case the mycelium reaches a height of about 2 mm., it tends to interweave into numerous little flexuous spires.

CONIDIAL STAGE.—As soon as the mycelium has thoroughly invaded the leaf tissue, conidia begin to appear in cavities among the palisade cells

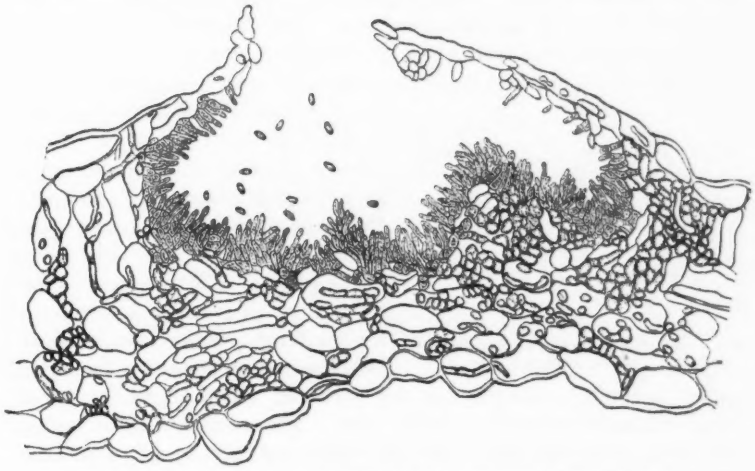

FIG. 1.—*Pyrenopeziza medicaginis:* Advanced stage of development of the conidial stage.

just below the epidermal layer of the upper surface of the leaf. At first these cavities are nearly spherical in form, but they rapidly increase in size, extending for the most part in the direction parallel to the surface of the leaf (fig. 1). Thus they become greatly broadened, and often much lobed and convoluted in cross section. The lining of the cavity consists of interwoven hyphæ or of a very thin layer of fungus tissue from which the conidiophores arise in a closely packed layer. Next to the epidermis there may be a few large, somewhat circular, dark-colored cells which do not form a complete protective layer. There is no regularly developed ostiolum, the opening being merely irregularly torn through the epidermis. Occasionally when the cavity is large and the weather is moist, the torn ends of the ruptured epidermis become recurved, which exposes the layer of conidiophores (Pl. 25). When the leaf tissue

has become largely replaced with the fungus stroma, it shrinks somewhat in thickness, and the large groups of conidiophores sometimes appear to be at the surrounding leaf surface or even raised above it.

The conidiophores have a characteristic bottle shape, measuring about 12 to 14 μ long and about 3 μ in diameter at the base, but much smaller in the upper third of the length (fig. 2).

The conidia vary in shape and size. The longer conidia are nearly cylindrical, very slightly bent, and with rounded ends. The shorter conidia often appear slightly shrunken at one end. They measure 5 to 9 by 2 to 3 μ, the larger part being 6 to 7 by 2.5 μ.

The larger part of the conidia found in cultures are borne in acervulus-like structures at the surface of the substratum or in spherical cavities within the substratum. The base of the acervulus, or the wall of the cavity, consists of closely woven hyphæ usually made up of short, rounded cells from which the conidiophores arise. But in addition conidia may be abstricted terminally, or even laterally, from hyphæ at any point on the surface of a culture; or hyphæ may give rise to either isolated or small groups of conidiophores which produce the spores (fig. 3). Sometimes it appears that an acervulus develops from a nucleus of a few conidiophores arising in a group. For instance, cultures from ascospores discharged on alfalfa stems sometimes show all gradations between a few scattered conidiophores, a larger, more matted group, and a somewhat raised acervulus. Spores may be produced from acervuli in such numbers that they exude in milky drops. The spores are indistinguishable from those produced on the leaf, except that occasionally somewhat larger individuals can be found, measuring up to 10 μ in length.

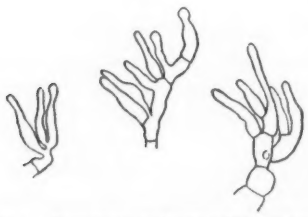

FIG. 2.—*Pyrenopeziza medicaginis:* Conidiophores from an alfalfa leaf.

In addition to what may be regarded as the normal conidia in culture, conidia-like structures are often formed on mycelium from ascospores discharged on agar to which little or no nutrient material has been added. The conidia-like structures are found submerged in the substratum, and are distinguished from normal conidia by the ovoid shape, slightly larger size, and by the fact that they are borne in groups (fig. 4).

ASCIGEROUS STAGE.—After the leaf tissue has been killed, when favorable conditions for further development of the fungus occur, small black stromatic masses emerge from the lower surface of diseased areas opposite the pycnidia. Later, they may appear in smaller number on the upper surface of the leaf, scattered somewhat beyond the black area on both surfaces. These black stromatic masses develop into apothecia. Before opening, the apothecium contains only a mass of vertical hyphæ with their upper ends free, and from these ends a few conidia may be

abstricted. When the apothecium is ready to expand, asci appear among the paraphyses, and the ascospores are soon mature and ready to be discharged (fig. 5).

The apothecia are sessile, 0.25 to 1 mm. in diameter, rarely larger. The outer wall appears black and is made up of thick-walled cells. The disk of the apothecium appears pale gray when expanded. The stromatic layer from which the asci arise is continuous with the stroma in the leaf, but it is composed of smaller cells rich in protoplasmic contents.

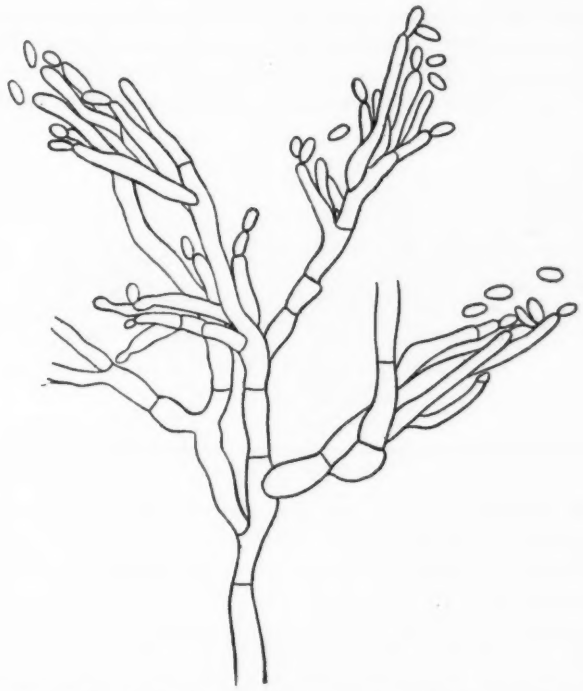

FIG. 3.—*Pyrenopeziza medicaginis:* Conidiophores from a culture at an early stage in the formation of an acervulus.

The paraphyses measure 50 to 80 by 2.5 to 3 μ. Occasionally they branch, in which case a septum may occur. The asci measure 60 to 75 by about 10 μ. The ascospores are slightly ovoid in shape and measure 8 to 11 by 5 to 6 μ. By far the largest number are 9 to 10 μ long.

PHYSIOLOGY

ISOLATION OF THE FUNGUS

For over a year after the yellow-leafblotch had first been observed, all efforts to isolate the fungus which appeared to be the cause were unsuccessful. All attempts to germinate conidia failed. Attempts to plate out

diseased fragments of the leaf after surface sterilization were unsuccessful at that time because bacteria or saprophytic fungi, which had entered the diseased tissue at a very early stage, quickly overran the slow-growing pathogen in culture. However, in October, 1915, the disease appeared on clean, vigorous plants in the greenhouse. Platings from these leaves gave the first success in isolation that was achieved. The fungus thus obtained grew very slowly, and produced conidia indistinguishable from those found on the diseased leaves. Like the conidia found on diseased leaves, these failed to germinate, and did not produce the disease when sprayed on alfalfa plants. From this time on nearly all the successful isolations have been made from plants in the greenhouse. However, late in the autumn of 1916, successful isolations were made from vigorous plants in a field near the laboratory where the progress of infection

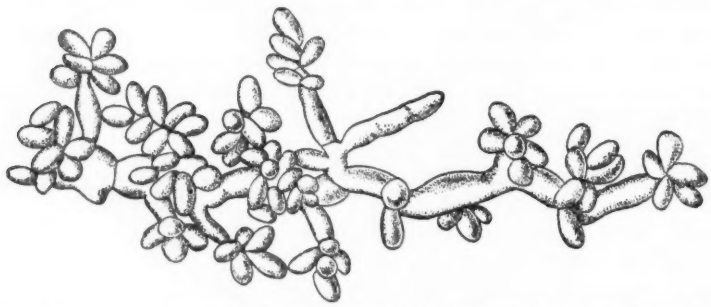

FIG. 4.—*Pyrenopeziza medicaginis:* Conidia-like structures which occasionally develop on mycelium from germinating ascospores.

could be watched and isolations made at the time of the first visible stages in the development of the disease.

The method followed in making these isolations was as follows. The diseased area at an early stage of development was cut from the leaf. The fragments were small—not more than 5 mm. long—and they were cut in such a way that the diseased area was not entirely surrounded by healthy tissue. Much time is required by the fungus in culture to cross even narrow bands of healthy tissue which may separate it from the surface of the substratum. The leaf fragment was then dipped into 50 per cent alcohol, and sterilized on the surface in a mercuric chlorid solution for from 1 to 1½ minutes. After washing, the fragments were placed singly on slopes in test tubes. Petri dishes are unsatisfactory, since they usually dry out before the end of the three weeks that are required for the fungus to grow sufficiently to furnish transfers. The culture medium most favorable for the development of mycelium appears to be potato agar.

After the ascogenous stage of the fungus had been found, efforts were made to make isolations from ascospores. But no practicable method

of obtaining cultures free from bacteria from ascospores from apothecia on dead leaves was devised. When plated out in agar, the ascospores refuse to germinate. When discharged on an agar surface, germination was not vigorous and bacteria were almost always present. A few germinating single spores were obtained free from bacteria, but they did not develop into cultures. In fact, no cultures from single spores of this fungus have yet been obtained.

Later, however, a method of obtaining apothecia under conditions of pure culture made it possible to secure cultures from ascospores. While isolations from the diseased plants in the greenhouse were being made repeatedly to determine if the fungus which had been obtained was constantly associated with the disease, various culture media were tried out. In the course of these experiments it was found that when agar with no

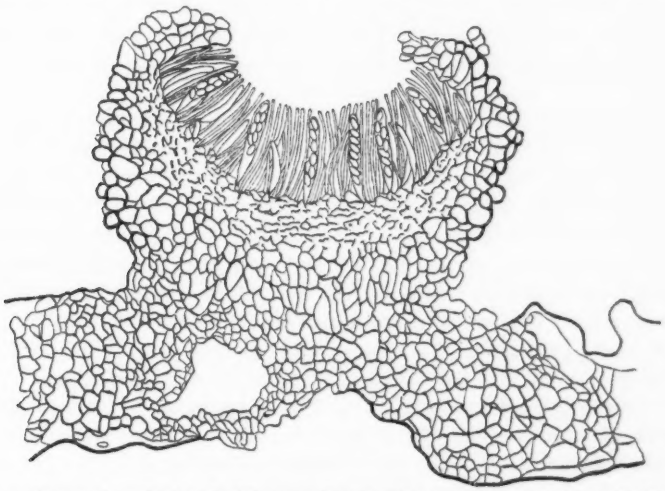

FIG. 5.—*Pyrenopeziza medicaginis:* Semidiagrammatic section of an apothecium. The tissue of the leaf has been largely replaced by the fungus hyphæ and stroma.

nutrient material added was used as a culture medium for isolation very little mycelial growth developed outside the diseased leaf fragment, but that in many cases apothecia were produced. A little less than half of the leaf fragments thus treated usually developed apothecia in about three weeks if kept at a temperature of 18 to 20° C. Above 22° apothecia occur less frequently and are not so well developed. On many of these leaf fragments conidia are not produced abundantly along with the apothecia. Thus, by selection, apothecia can be obtained which will discharge in great abundance spores free from conidia or the spores of any foreign organism. From these spores discharged on an agar surface cultures can easily be obtained. The peculiar behavior of these cultures which distinguishes them from those obtained from mycelium in the leaf tissue will be noted in the following section.

PRODUCTION OF APOTHECIA IN PURE CULTURE

The cultures which are obtained from ascospores discharged on an agar behave in early stages of development very differently from cultures which have been obtained from mycelium in the leaf tissue. Mycelium develops very slowly. Even when the agar surface has been abundantly strewn with spores, mycelium does not become visible until after a week or 10 days. It grows almost wholly within the surface of the substratum and soon unites to form a crust that varies in color with the nutrient material furnished. Enormous numbers of conidia are produced that sometimes cover the crust with a slime. If this crust covers the entire surface of an agar slope, aerial mycelium is rarely developed in visible amount. Such a culture appears very different from one developed from mycelium from the host tissue. However, if a fragment of this crust is cut out and placed on a fresh agar surface, mycelium will emerge from its edge; and in the course of a few weeks transfers can be made which are indistinguishable from transfers from cultures obtained from host tissue.

This peculiar crust produced by mycelium from ascospores was not carefully studied until the discovery had been made that one such culture was producing apothecia. Apothecia from this and other sources were immediately used to start other cultures to determine the conditions requisite for the development of the ascigerous stage. Since the supply of ascospores was somewhat limited, and since the time required for the formation of apothecia under the best of conditions found is from five to eight weeks, it has not been possible to carry this part of the work to a satisfactory conclusion. In no case have apothecia appeared in all the cultures of a set that have been held under identical conditions. This may be due in part to the fact that it is not possible to get two slopes "seeded" with even approximately the same number of spores. Thus far the ascogenous stage has been produced in 20 cultures.

The best culture medium for this purpose appears to be oatmeal agar, though apothecia have been produced on potato agar and alfalfa stems. Spores may be discharged on a layer of clear-water agar in a petri dish, where their number and distribution may be observed before transfer with the substratum to the agar slope in the test tube; or they may be discharged directly upon the agar slope. The latter method appears to be somewhat better.

With regard to temperature and light, by far the best success has been attained in cultures exposed to weak light in a room which normally maintains a temperature of about 21° C., but which at several times during the growth of the cultures fell to 14° to 16° for several days. Continued low temperature does not appear to favor the production of apothecia. Cultures grow only slightly at 8°. Cultures held at constant temperatures below 14° have shown no indication of producing apothecia

after three months of incubation. Thus, although apothecia have been produced on artificial media in sufficient amount for inoculations and other experimental work, no method of producing them with definite certainty and in large number has been devised.

Description of Cultures on Special Media

It will be seen from the foregoing that a study of the fungus on each culture medium might properly include a comparison on that medium of cultures from three sources: (1) Ascospores discharged on that substratum, (2) transfers of mycelium developed from ascospores, and (3) transfers of mycelium obtained from plating out diseased leaf fragments. The last should be included because, inasmuch as it produces no spores that are capable of infecting the host, its identity as the pathogenic organism can only be shown by demonstrating its identity with cultures derived from ascospores which, beyond question, belong to the pathogene. Of course, it would be anticipated that the cultures from these sources behave alike, and comparisons have shown that this is the case, the only difference being that cultures from ascospores sometimes appear to have slightly greater vigor. Since cultures appear to behave somewhat differently after they have been kept on culture media a year or more, comparisons are made of only recent isolations. Such notes of the development of the fungus from ascospores will be made as appear significant.

The following descriptions are made only for the purpose of assisting in the identification of the fungus.

Cultures on potato-dextrose agar (slanted tubes)

From transfers of mycelium.—The typical form of the culture on this substratum as developed in three or four weeks is a raised knob 3 mm. or more high, surrounded by a thinner raised growth that decreases in thickness to the edge. Four to six weeks at optimum temperature are required for the fungus colony developing from a small transfer to extend entirely across a slope in a 15-mm. test tube. At the edge of the growth the submerged mycelium is usually very slightly in advance of the aerial mycelium. The short, matlike aerial mycelium of ascending hyphæ varies greatly in color, tending to become darker at high temperatures and remaining white at low temperatures. Ordinarily it is white or gray, often with the admixture of a slightly pink tint.

Conidia can almost always be found scattered about on the aerial mycelium at various stages in the development of the culture. After the cultures are 3 or 4 weeks old, conidia are produced in more or less definite acervuli on typical conidiophores. These acervuli may be scattered about over the culture or produced in groups on a black stromatic base.

FROM ASCOSPORES.—Ascospores discharged on potato agar develop a yellow color much like that described later for oat agar, but conidia are produced early in greater abundance, and when these ooze out, the culture becomes a dirty gray. Aerial mycelium is absent except on that part of the culture which has begun to dry out.

CULTURES ON OATMEAL AGAR (SLANTED TUBES)

FROM TRANSFERS OF MYCELIUM.—Cultures on oatmeal agar resemble in form those on potato agar, except that the growth is not raised and therefore the central knob is lacking. Growth is a little less vigorous. Instead of a pink color, occasionally a yellow color is developed in a portion of the culture, which becomes matted and wet as though bacteria were present. This indicates the abundant development of conidia from the mycelium without the formation of a definite acervulus.

FROM ASCOSPORES.—Cultures from ascospores discharged on oat agar are easily conspicuous by reason of the yellow color produced, which is of much the same character as that produced on the living leaf. The intensity of the color depends in part on the number of the fungus colonies which are developed. If they are closely crowded, in the course of two or three weeks the color is ochraceous orange. If the colonies are less numerous, the color is duller, becoming a clay. Conidia are produced more abundantly from the colonies with the larger amount of surface space, being less crowded.

CULTURES ON DEXTROSE AGAR (2 PER CENT DEXTROSE AND 2 PER CENT AGAR)

FROM TRANSFERS OF MYCELIUM.—Growth is very meager. The larger part of the mycelium is submerged in the substratum, and is dark olive in color. Aerial mycelium is white. No conidia have been noted, even after a growth of six weeks.

CULTURES ON STERILE ALFALFA STEMS

FROM ASCOSPORES.—The stems used for these cultures were gathered standing through the snow in midwinter. When ascospores were discharged on these stems, a growth resembling that on oat agar was produced. In two or three weeks the color on the more moist portions of the stems was cinnamon-buff. On dry portions there was a little white mycelium. Conidia are produced in great abundance. An illustration of a way in which these conidia are occasionally borne on these stems has already been given (fig. 3). The mycelium penetrates only a few outer layers of cells of the substratum.

SPORE GERMINATION

GERMINATION OF CONIDIA

The first attempts to germinate conidia were made for the purpose of isolating the fungus. But although plates were poured repeatedly, no spore that could be identified as belonging to this fungus was ever observed to germinate.

When pure cultures had been obtained, efforts were resumed under more favorable conditions. Germination was attempted in poured plates, on an agar surface, in distilled water, and in several liquid media at temperatures within the range through which the mycelium of the fungus grows. A few doubtful cases of germination have been noted in which it could not be determined whether a structure attached to a spore was an attached conidiophore or a germ tube. But since the structure did not develop beyond the length of a normal conidiophore, it was assumed to be such.

The nearest approaches to germination have been observed in the case of spores flooded on a plate of potato agar, the culture medium upon which the fungus grows best. In early tests it appeared that a single spore sent out a lateral germ tube about 20 μ in length. Later, occasional spores on this substratum have shown a slight bulging of the cell wall, but no well-defined germination has taken place. In order to allow conidia a long time in which to develop fungus colonies, they have been flowed in a water suspension on agar slopes in test tubes. The free water was poured off and the tubes were set aside for several weeks; but no fungus colonies developed upon them.

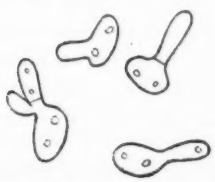

FIG. 6.—*Pyrenopeziza medicaginis:* Germinating ascospores.

Conidia upon dead leaves have been included in germination tests, but not enough of over-wintered conidia have been secured for this purpose. These results, together with the fact that no successful inoculations with conidia have been made, make it seem unlikely that they germinate often, at least not in significant numbers.

GERMINATION OF ASCOSPORES

Ascospores of *Pyrenopeziza medicaginis* germinate readily on an agar surface, or in distilled water, but very poorly, if at all, when they are submerged in poured agar plates. The percentage of germination is very variable, usually being rather low, from 25 to 30 per cent but sometimes being as high as 80 per cent. No explanation for this variability can be offered. The spore germinates from any part of its circumference with little apparent preference for the side or the end (fig. 6). The germ tube is soon cut off by a septum. Other features of germination will be discussed under temperature relations.

RELATIONS OF TEMPERATURE

GROWTH OF MYCELIUM

In order to determine the optimum temperature for the development of mycelium, transfers were made on potato agar and placed at temperatures ranging from 2° to 29° C. These were kept under observation for

six weeks. At the end of this time the growth in the cultures at the two extremes, 2° and 29°, was barely perceptible. The best growth was made at temperatures between 16° and 25°, although at the lower temperature it was somewhat retarded. Between 2° and 16° there was a remarkably regular retardation of growth in proportion to the lowered temperature.

GERMINATION OF ASCOSPORES

Three tests of the time required for the germination of ascospores at different temperatures have been made. As a result of preliminary trials, the following method of making germination tests has been found the most satisfactory. The leaf fragment or culture bearing apothecia which are discharging spores is placed in the cover of a petri dish in which a thin layer of 2 per cent water agar has been placed. Since the act of transferring the culture to the petri dish often affects the rate of discharge of ascospores for a time, at least 12 hours should be allowed to elapse before spores are taken for germination test. Then the cover of the dish is turned so that the spores fall in a new place. When a sufficient number of spores have collected (from one to three hours will be required), the area on which the spores are collected is marked with a wax pencil, and the cover is transferred to another dish of agar if more test plates are needed. The plate on which the spores have been collected is then placed at the desired temperature. The spores can easily be observed through the bottom of the plate under the low power of the microscope.

This method is open to the objection that some of the spores are exposed on the plate for one or more hours at room temperature before they are placed at the desired temperature. This would be a serious matter if the spores germinated quickly; but since nearly 12 hours are required for complete germination, it does not appear to be a large factor.

The results of the tests are compiled in Table I. At almost all of the temperatures used at least three trials have been made with spores from different cultures. The results of the trials have not been divergent outside the limits of error incidental to such tests. In Table I the numbers in the columns under the length of time during which the spores had been kept at the given temperature represent the length of the germ tube at that time in relation to the greatest diameter of the spore. This number is obtained by estimate, and not by measure. In making the estimate after the longer periods of time, the shorter germ tubes which have apparently ceased growth are disregarded, and only the length of the longer ones is considered. It may be added here that at the extremes of temperature, where germination does not proceed far, the percentage of germination is also greatly reduced.

From this table it appears that the optimum temperature for spore germination is between 12° and 26° C.

TABLE I.—*Time required for the germination of ascospores of Pyrenopeziza medicaginis*

Temperature.	Estimated length of germ tube (relative to greatest diameter of spore) at incubation periods of—				
	12 hours.	24 hours.	36 hours.	48 hours.	72 hours.
°C.					
2–3					0.5
4–5				0.5	1
6			1	2	3
8		1	1–2	2	3
12		1–1.5	1–2	2	3
14	1	1–2	2–2.5	3–4	
18	1	1–2	3	4–5	5–6
21–22	1	1–1.5		2–3	
26	1	1–2	2	3	3–4
29[a]					
30[a]					

[a] Germinating spores rare; growth not continued.

PATHOGENICITY

INOCULATIONS WITH CONIDIA

Inoculations with conidia were begun when the disease was first found in order to determine if the conidia found so abundantly in diseased areas belonged to the organism causing the disease. In no case was any degree of success in producing infection obtained. When the fungus had been cultured and had been shown to be the cause of the disease, further efforts to secure infection with conidia were made. Various methods of making inoculations were followed. Spores from diseased leaves or from pure cultures were sprayed over the plants in a water suspension. The plants were kept in a moist chamber for at least 24 hours. In order to get large numbers of spores on selected leaves, diseased leaf fragments from which conidia were oozing were placed on moistened alfalfa leaves for about 24 hours in a moist chamber. When these leaves finally became dry, the mass of conidia appeared as a slight white incrustation. This likewise failed to produce any diseased condition. In order to make conditions more severe, other leaves were slightly wounded by abrading the cuticle before inoculation was made. This likewise was unsuccessful. No conditions were found under which these conidia were viable and capable of producing infection. However, negative evidence of this kind must be kept open to question.

INOCULATIONS WITH ASCOSPORES

In contrast with the results obtained from inoculations with conidia, no inoculation with ascospores has yet failed to give a larger or smaller percentage of infection. A few of the earlier inoculations in the greenhouse in the late summer were not absolutely conclusive, because at that time all plants showed a few infections, probably from spores blown in

from an alfalfa field close beside the greenhouse; but during two winters all plants in the greenhouse except those inoculated have remained free from the disease.

Ascospores for inoculation have been obtained from dead leaves from the field, from apothecia on alfalfa leaves under conditions of pure culture, and from apothecia produced on culture media. The following method of inoculation, which was first devised to secure the discharge of ascospores from dead alfalfa leaves upon the leaf to be inoculated with the least possible danger of transferring conidia or other spores, has been used in many inoculations with this fungus. For this purpose a small glass dish is made by cementing a heavy cover glass to one side of a glass ring about 15 mm. in diameter and 6 mm. deep. In the bottom of the dish thus formed is placed a fragment of dead leaf bearing apothecia which appear mature. If dry leaves from the field are used, they should be kept thoroughly wet for at least 12 hours before being used to obtain the best discharge of spores. These leaf fragments remain firmly attached to the bottom of the glass dish when kept wet. In order to determine whether spores are actually being discharged and how abundantly, the glass dish is then inverted on a thin layer of clear-water agar in a petri dish. If, after an hour, very few or no spores are found on the agar, the fragment is discarded and a new one substituted. The agar plates are kept to ascertain whether the spores are viable.

The dishes containing material that is discharging a suitable abundance of spores is then placed over an alfalfa leaflet which is supported so that the petiole is not greatly bent. The leaf is previously wet with a fine spray, or rubbed slightly to cover it with a thin film of water, or sometimes it is sprayed after the spores are applied. After the dish has been inverted over one leaflet for an hour or more, it is transferred to another. The leaves thus inoculated are marked. It has been found that apothecia on dead leaves can usually be depended upon to maintain a discharge of spores for at least 18 hours. The inoculated plant is kept in a moist chamber for at least 12 hours after the last set of leaves has been inoculated.

Another method that has also been used consists of placing the material from which ascospores are being discharged in the top of a bell jar over plants which are well sprayed. This method has produced very abundant infection on young plants, especially on vigorous seedlings; but with the old plants infection is usually meager. Apparently, young tender leaves are much more easily infected than older ones, although infection may take place at any age.

One of these inoculations with ascospores from dead leaves made on November 4 and 5, 1915, is summarized in Table II as the result appeared on November 22. Several of the inoculated leaves had fallen at this time and are not included in the results.

TABLE II.—*Result of the inoculation of alfalfa leaves with ascospores of Pyrenopeziza medicaginis from dead leaves*

Plant No.	Conditions of inoculation.	Number of inoculated leaves.	
		Diseased.	Not infected.
1.....	Plant growing vigorously, leaves young, sprayed.....	10	11
2.....	Very vigorous plant, leaves young, rubbed till surface was uniformly wet.	6	0
3.....	Very vigorous, leaves rubbed until wet, inoculated on lower surface.	5	1
4.....	Plant not growing, leaves not young, but healthy. Surface rubbed.	8	6

Results of this kind have been obtained repeatedly with inoculations from ascospores under conditions of pure culture, as well as from dead leaves. When only very young vigorous leaves are chosen for inoculation and the material used discharges spores in abundance, a 100 per cent infection can usually be obtained. Either the upper or lower surface of the leaf can be infected.

The failure to get a 100 per cent infection in all cases appears to be due in part to the variable and often very low percentage of germination of ascospores. It has been noted especially with ascospores discharged from leaves that not more than 1 or 2 per cent of the first spores to be discharged after the leaf is soaked fail to germinate more, while 25 per cent of those discharged a few hours later may germinate. But sufficient observation of germination under these conditions has not been made to warrant the opinion that this is a general rule.

The time required for the yellow-leafblotch to appear after inoculations in the greenhouse is approximately two weeks. In case a great number of viable spores has been placed on the leaf, the yellowing begins a few days earlier; but if the number of spores is small, giving rise to but a few penetrations, the yellowing is delayed until a few days later. Heavy infection may produce yellowing prior to the development of pycnidia, and, in fact, the leaf may be killed before pycnidia can be formed. When infection is light, a leaf may not show a yellow blotch until very shortly before the pycnidia are formed. Under greenhouse conditions the diseased leaf tissue usually dries out so early that no apothecia develop. Only in a single case, where the inoculated plants were growing in a dense mat that maintained a constant condition of high humidity, did apothecia appear.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS AND CONTROL MEASURES

PRODUCTION OF CONIDIA

As has been described previously, conidia are formed in the pycnidia sometimes even before the symptoms of the disease are conspicuous. They are usually abundant by the time the yellowing is distinctly visible. It appears that they are produced in greatest amount while the leaf tissue surrounding the pycnidia is still alive. When the diseased area dies and dries, conidia production appears to cease. When the entire leaf is dead the conidiophores are usually found disorganized, though the cavity may still be filled with spores. But it is doubtful if the production of conidia always ceases with the death of the leaf. In a few instances, while examining structures that appeared like undeveloped apothecia on dead leaves in the summer; and in one instance in the spring, these structures were found to be filled with typical conidia borne on conidiophores. Since conidia production has been found under such circumstances but two or three times, it is assumed that it is not a frequent occurrence. There has been no opportunity to determine whether these conidia are capable of germination. Ordinarily by the time that apothecia are mature the conidia have completely disappeared from the diseased leaf.

PRODUCTION OF ASCOSPORES AND OVERWINTERING

Since the experimental evidence indicates that the ascospores are the only source of infection, a careful study of the conditions under which they are produced becomes important. Apothecia containing spores that will be discharged after a few hours of soaking have been found most abundantly in the autumn, and even in the early winter, especially in the late growth of alfalfa which has become well infected before killed by frost. If these leaves are not subjected to frequent wetting, spores will be retained in a viable condition, at least over the winter. This has been shown by the fact that collections made in October, 1915, and wintered in a cage out of doors were used successfully to produce infection the following February, and spores were discharged from this material as late as the following August. But in the field the spores formed in the fall appear to be discharged long before spring, and no new spores are formed until after a period of warm weather. On May 1, 1916, apothecia were collected at Madison, Wis., on overwintered leaves in the field, but no mature spores were found in them until they had been kept four or five days in a damp chamber at room temperature. On May 10 several collections of apothecia containing spores were made. By May 28 the disease was abundant in the localities where the apothecia had been found on May 10. On the latter date a few apothecia with mature

spores were found on diseased leaves of a plant in a protected location which had grown more rapidly than most plants in the open field and where infection must have occurred earlier. From this time on infection from overwintered material was reenforced with infection from ascospores from diseased leaves of the current season's growth.

During the summer, apothecia were found in great abundance in a field left uncut for seed production. During the hot weather of July and August these failed to open when soaked, and appeared to be entirely lifeless. Yet, after the crop had been cut and the rains had come and a new crop had sprung up, it was found to be very heavily infested with the leafblotch. In late October apothecia producing spores in great abundance were found in these fields, and the late autumn growth showed much of the disease.

It is of interest to note that infected leaves which remain attached to the plant appear to be much more favorably situated for the production of apothecia than those which early fall on the ground. This is true both in summer and in winter. Infected leaves which are early beaten to the ground by rains rarely develop apothecia. The overwintered leaves which produce apothecia most abundantly are those which have remained attached to standing stems until early spring.

Thus, it appears that apothecia readily survive the winter; possibly others are developed from the stroma in the leaf in spring. The cold weather of early spring appears to be unfavorable for abundant ascospore production; and the hot, dry weather of midsummer also has an inhibiting effect. So far as observation has gone, ascospores appear to be produced in sufficient amount to account for all infections that have been observed.

PENETRATION OF THE HOST

In spite of considerable effort to determine the method by which the germ tube from the germinating spores enters the leaf, only about a dozen instances of penetration have been observed. Apparently, after the germ tube has entered the leaf, the spore breaks away from the leaf easily, and the relation of the mycelium to the exterior of the leaf is hard to trace. But in the few instances which have been observed, the germ tube has penetrated directly through the cuticle either immediately beneath or close by the spore. In a majority of cases the spore has been located at the junction of two epidermal cells, but in others the spore was near the center of an epidermal cell. None of the germ tubes which have grown out over the surface of the leaf have been seen to enter. No case of entry through a stoma has been observed.

METHOD OF DISTRIBUTION

Up to the present the disease has been observed closely in but few localities; in Wisconsin it has not been seen in newly seeded fields which are several miles from old fields where the disease is known to

occur. It has been observed the first year in newly seeded fields in close proximity to old fields where the disease occurs. Thus, there is no evidence either from the study thus far made of the life history of the fungus or from field observation that indicates that the disease is carried by properly cleaned seed. But it does appear likely that the ascospores are blown at least short distances by the wind. It is also clearly evident that any infected alfalfa hay or débris might easily convey apothecia which would become a source of infection under suitable conditions.

CONTROL MEASURES

No experiments have been conducted to determine the efficiency of possible control measures. If, as now appears, the only source of infection is the ascospores, cutting the alfalfa before these are mature should greatly reduce the disease. In fact, so far as the writer has observed, this is the case. In fields which make a vigorous growth during the entire season and which are cut for hay at the stage usually recommended, this leafblotch is never important. But if for any reason an infected field is allowed to remain uncut for an unusually long period, especially in the cool, moist weather of spring and autumn, the disease becomes abundant and destructive, provided a source of infection is present. Lodged plants which have escaped cutting, or plants left standing in fence corners, may become important sources of infection at this time. Uncut plants of this character also provide excellent facilities for the overwintering of this fungus, as well as several others, and should be carefully eliminated wherever the disease is troublesome.

SUMMARY

(1) The disease of alfalfa here described as the yellow-leafblotch is one of considerable economic importance which has been recognized in America only during the last three years (1915–1917), although it has long been known in Europe.

(2) The yellow-leafblotch occurs in important alfalfa-growing regions from New Jersey to Oregon, and at least as far south as Tennessee.

(3) The injury is brought about, either directly by a slow killing of the infected leaves, or indirectly by furnishing easy access to the weakened leaves for other organisms.

(4) The causal organism (*Pyrenopeziza medicaginis*) is a fungus which produces, first, a conidial stage on the living leaves and later, ascigerous stages on the portion of the leaf which has been killed.

(5) The fungus has been grown in culture, where both the conidial and ascigerous stages have been produced.

(6) Infection appears to take place only from ascospores, which upon germination are able to penetrate the epidermal cells of the leaf. The viability of the conidia has not been conclusively demonstrated.

(7) The fungus overwinters on dead leaves which were infected the previous autumn.

(8) Cutting infested fields before the ascigerous stage of the fungus has developed on infected leaves appears to hold the disease in check.

(9) Control measures when necessary must apparently be developed as a method of sanitation that will remove the dead leaves on which the apothecia develop.

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PLATE D

Alfalfa showing the yellow-leafblotch.

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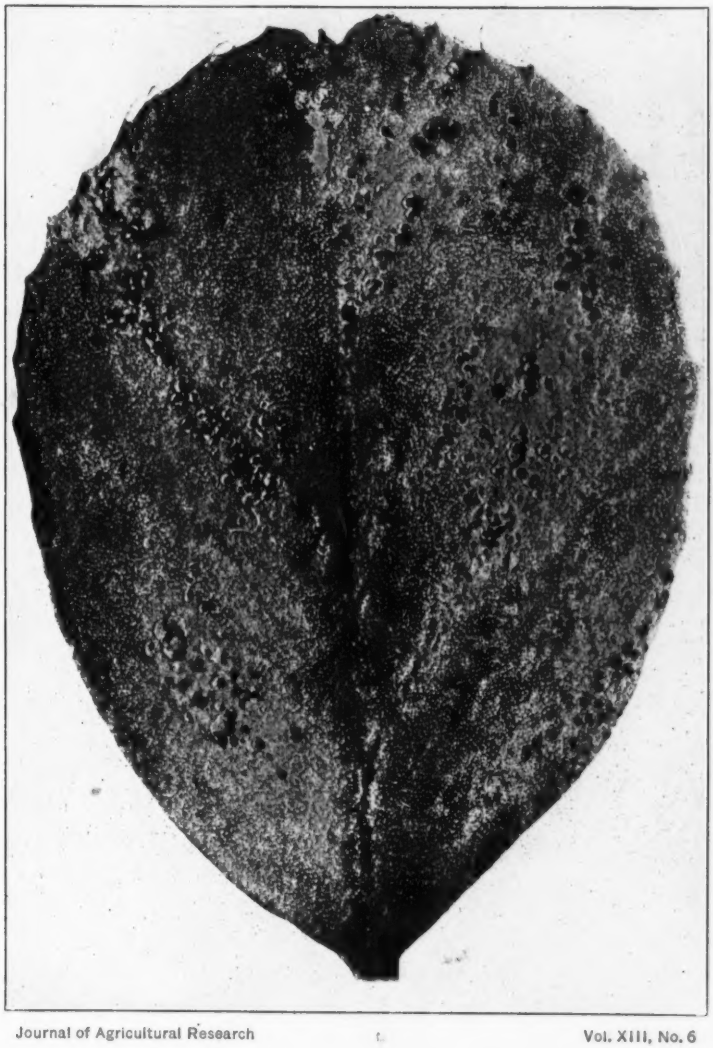
PLATE 25

Pyrenopeziza medicaginis: Conidial (Sporonema) stage on a leaflet of alfalfa.



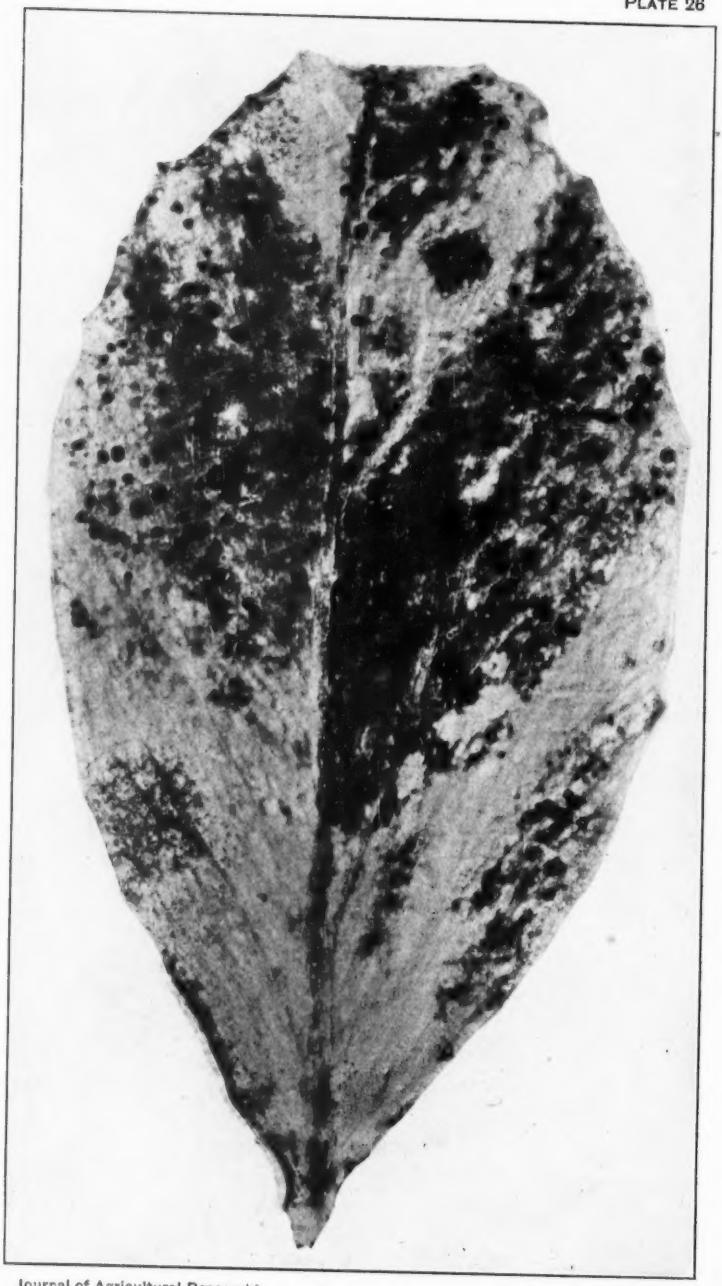


PLATE 26

Pyrenopeziza medicaginis: Apothecia on the lower surface of a dead leaflet of alfalfa.

AN UNDESCRIBED CANKER OF POPLARS AND WILLOWS CAUSED BY CYTOSPORA CHRYSOSPERMA

By W. H. Long

Forest Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the semiarid regions of the Southwest tree growth is not favored by climatic conditions as it is in the East. This is especially true of ornamental and shade trees suitable for homes, streets, and parks. Several species of poplar (*Populus* spp.) and willow (*Salix* spp.) are able to grow into beautiful ornamental and shade trees under the adverse conditions in many of the Western States. Since there are so few species of shade trees capable of growing in semiarid regions, it is of great importance that special attention be given to any disease which may attack these trees.

From time to time specimens of diseased bark from various species of poplar have been received by the writer from several Western States, accompanied by statements that the trees were being killed and requests for information as to the cause of the trouble. On account of the seriousness and wide distribution of the disease, investigations as to its cause and control were undertaken. This article gives the results of these investigations.

The disease has been found at various altitudes, ranging from 1,000 to 8,000 feet. It is common in the semiarid regions of the Southwest on various species of poplar, especially when used as shade or ornamental trees, and occasionally on willows.

DESCRIPTION OF THE DISEASE

This disease occurs in the form of lesions or cankers on the trunks and large limbs of affected trees. It is also found attacking the small branches and twigs. These are usually killed without forming any definite canker.

The lesions caused by this disease resemble what is often called "sunscald" on the trunks of fruit trees. The diseased bark is gradually killed in more or less circular areas. Young infections on smooth-barked shoots can be recognized by the presence of brownish, shrunken patches. However, these differ but little in general appearance from the surrounding healthy bark.

The area invaded by the fungus may be fairly regular or very irregular in outline. The diseased area gradually enlarges until the trunk or

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branch is entirely girdled and killed. The fungus often enters the dead tips of twigs or small branches. It then gradually kills the invaded branches back to their juncture with a larger branch or with the trunk. The fungus then develops on the large branch or the trunk a more or less circular canker around the base of the dead twig. Reddish fruiting pustules may appear on the dead areas near the edge of the canker; or in smaller branches they may appear over the entire surface killed by the fungus. The inner bark of the diseased areas gradually turns black and often gives off a foul, salty odor. The sapwood, especially the medullary rays, is also diseased and is stained a watery, reddish brown (Pl. 27, A), and the heartwood is sometimes discolored. Trees 3 to 6 inches in diameter which are severely attacked have but a few, sickly looking leaves (Pl. 27, B), and such trees usually die in two or three years. Trees which have been rapidly girdled by this disease often develop sprouts from the roots (Pl. 27, C) in a manner similar to that of the chestnut when attacked by the chestnut-blight fungus (*Endothia parasitica*). These suckers are usually ultimately killed by the invasion of the fungus from the old diseased parent stem.

Canker often attacks the old trunks and large branches of various species of poplar. In such cases the fungus causes but little, if any, perceptible change in the outward appearance of the bark. The attacked area is ultimately killed, and the typical dark-red spore horns of the fungus which causes this disease are developed in the fissures of the bark.

The silver-leaf poplar (*Populus alba*) when attacked by this disease dies branch by branch, since the disease usually enters the tips of the branches in the top and gradually works downward. The cankers found on the branches of this species usually extend from one to several feet farther on the under side of the branch than on the upper side. When the canker reaches the trunk of the silver-leaf poplar, it usually travels more rapidly longitudinally than transversely. This results in long, narrow, dead areas extending often for several feet down the tree. Finally the upper portion of the crown is killed, just as the individual branches were. When a tree is slowly killed from the top downward, few, if any, suckers are developed from the roots.

The dead areas produced on trees by this disease finally develop characteristic spore horns consisting of irregularly twisted threads (Pl. 27, D), which are often flattened, ranging in color from grenadine red[1] to English red. The spore horns when first formed are soft and sticky, but they dry rapidly and become hard and brittle.

On the young branches and small trees of the aspen (*Populus tremuloides*) the typical lesions or cankers of this disease occur, but on the large, old aspens another type of canker is common in the mountains of Arizona and New Mexico. These cankers are perennial and of much

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

slower growth. They usually originate from some wound in the trunk or from a dead branch. The disease first forms a circular dead area on the trunk around the base of the dead branch or around the wound through which it entered. The tree then attempts to limit the disease by developing a ring of callus around the infected area. The mycelium of the fungus, however, gradually grows under this callus and kills a new zone of tissue. This process is repeated year after year until there is formed a large canker consisting of successive rings of dead tissue. The old dead bark finally separates from the sapwood, leaving the dead area more or less exposed. The surface of this dead sapwood shows the concentric rings of dead callus which formed annually in the attempt to check the disease. No fruiting bodies of any kind have been found associated with these lesions, and it is very doubtful if this peculiar type of canker is caused by the disease discussed in this article.

Willows have also been found attacked by this canker disease, e pecially the weeping willow (*Salix babylonica*), which is often planted as an ornamental and shade tree. The willows are killed in much the same manner as the poplars

THE FUNGUS

This canker of poplars and willows is caused by a definite species of fungus which grows as a parasite in the bark and to a limited extent in the sapwood of infected trees. The causative organism is *Cytospora chrysosperma* (Pers.) Fr., since inoculations of this fungus made into healthy poplars have produced the typical cankers, and pure cultures of this organism have been reisolated from the cankers thus produced.

After the mycelium of the fungus has been growing for several weeks in the bark, it forms fruiting pustules or pycnidia. These pycnidia produce a large number of curved, hyalin, 1-celled spores which are extruded from the pycnidium in the form of threadlike irregular coils. These are called "spore horns" or "tendrils" (Pl. 27, D). The development of the spore horns is not limited to any special season, but may occur during any month of the year. The production of pycnidia seems to be limited to the bark, at least in the semiarid regions of the western United States, since no evidence of fruiting bodies of the fungus has been found on decorticated limbs, trunks, or stumps of affected trees.

The pycnidia are not evident on the surface of the bark until after the spore horns have been dissolved and washed away by rains; then the dead bark shrinks and the mouths of the pycnidia become evident (Pl. 28, A). Only the pycnidial stage of this fungus is known.

INVESTIGATIONS AS TO THE CAUSE OF THE CANKERS

Pure cultures of *Cytospora chrysosperma* were made by means of single spore colonies from spore horns obtained from *Populus wislizeni* and *P. alba*. Inoculations were made on small bushes of *P. alba* with material

from two sources: (1) Spores from spore horns and (2) spores from pure cultures. The typical lesions of this disease were produced by both methods.

Three series of inoculations were made under control conditions in the laboratory at Albuquerque, N. Mex., as follows: September 7, 1915, September 22, 1916, and December 21, 1916. In each series 12 plants were inoculated with the spores of *Cytospora chrysosperma*, and 6 plants were used as controls. In the first and third series all of the plants inoculated with the fungus developed the typical lesions of this disease. In the second series 9 out of the 12 plants were infected, but all of the control plants in each of the three series remained healthy. All inoculations were made by incisions through the bark (about $\frac{2}{3}$ inch long) with a sharp, sterile scalpel. The spore horns were first dissolved in sterilized water, and this water was introduced into the incisions. In inoculations from pure cultures, the spores used came from soft spore horns grown on the surface of petri dishes. These spores were not put into water, but a small quantity of the spore mass was introduced with a sterile scalpel into the incision. Incisions which were not inoculated with the spores were made as controls. All incisions, including the controls, were immediately wrapped with wet absorbent cotton, which was left on the inoculated plants for 10 days. All the plants were kept in the laboratory and watered at the ground surface. The stems and tops of each plant therefore never had any water on them after being inoculated. By this method all chances of outside contamination were practically eliminated.

In 10 to 15 days after the plants had been inoculated, typical lesions of this disease began to develop in the shape of shrunken, dying areas about 4 mm. wide at the points of inoculation, but the control plants were healing normally. These lesions gradually spread until the stem was finally girdled at the point of inoculation, and the upper part killed. The first evidence of infection was usually a shrinking of the bark on the diseased area; later, on very young twigs the diseased bark turned black. Stems 6 to 12 mm. in diameter at the point of inoculation were entirely girdled in from two to four months. In some instances sprouts were developed below the girdled areas (Pl. 28, B) on the inoculated plants. In nature trees 1 to 3 cm. in diameter have been found which had been entirely girdled and killed by *Cytospora chrysosperma* in one year. On account of the small size (6 to 12 mm. in diameter) of the plants used in the laboratory for inoculating experiments and the dryness of the air indoors, the plants inoculated and killed by the fungus did not form any spore horns. The fungus was reisolated, however, from the cankers by taking small pieces of the inner bark at the boundary between the sound and diseased areas and placing these pieces in artificial culture media. Five or six weeks after the media had been inoculated with the diseased bark, the typical spore horns of *C. chrysosperma* began to develop in the tubes and petri dishes.

CULTURAL STUDIES

MEDIA.—*Cytospora chrysosperma* has been grown by the writer on two culture media: Corn-meal and malt agars. This fungus will probably grow on any of the usual media, to judge from the growth made on these two.

ISOLATION.—The fungus is easily isolated by removing under sterile conditions a small piece of the diseased tissue of the inner bark and transferring it to agar tubes. It can also be isolated by means of the pycnospores on agar slants or by the poured-plate method.

PHYSIOLOGICAL CHARACTERS

Certain characters are common to the growth of this fungus on corn-meal and malt agars. The most characteristic reactions were obtained with pure pycnospore cultures on 2 per cent malt agar, +7 (Fuller's scale).

On this medium the following characters develop when streak cultures are made:

The mycelium begins along the streak as a white cottony growth which spreads rapidly toward the sides of the tube. In seven days at ordinary room temperature the white color of the aerial mycelium gradually changes to a light buff, while in 10 days the submerged mycelium seen in mass is turning black. In 16 days the aerial mycelium is cottony in character and covers the entire exposed surface of the agar slant. It now varies from light buff to warm buff in the upper portion of the slant, while the lower portion is turning to a neutral gray and is becoming matted and adherent to the surface of the agar. The submerged mycelium is now black in mass and shows as a very distinct and characteristic border along the edge of the agar. Twenty days after the tubes had been inoculated, very small pycnidia, 0.4 to 1 mm. in diameter, were developed at the edge of the cultures next to the glass in the petri dishes and test tubes which were exposed to strong, diffused light. These small pycnidia contained typical pycnospores, which were discharged against the glass sides of the tubes in place of into the air. The test tubes of malt agar 20 to 25 days after inoculation show a very characteristic color reaction when the tubes are viewed from the rear of the slant—viz, the agar has retained its normal color except at the edge of the agar and air; here is seen a black layer of submerged mycelium bordered above by a layer of white to warm-buff aerial mycelium.

As the cultures in the tubes grow older, much of the aerial mycelium gradually becomes wet, mats together, and adheres to the surface of the agar, varying from mouse-gray to black in color; here and there elevated patches of white to light-buff mycelium remain (Pl. 28, C). These elevations are 3 to 6 mm. in diameter, hemispherical in shape, and contain the pycnidia. About 30 days after inoculation large typical pycnidia mature and begin discharging spores (Pl. 28, C). On plate cultures of malt agar this fungus has the same general characters of growth, except that the

aerial mycelium after about three weeks has changed to a mouse-gray color in the center of the plate, but at the margin it still retains its white to light-buff color. The entire surface of the plate is now covered with many elevated pustules 4 to 7 mm. in diameter. These develop pycnidia later if sufficient moisture is available. These pycnidia extrude a mass of pycnospores which is orange-chrome when fresh and soft, but which changes to English red on drying and hardening. These spore masses rarely form long spore horns, but usually remain as large orange-chrome drops at the point of issuance. However, if the air in the plate is dry, the typical spore horns of *Cytospora chrysosperma* are developed. Only a few pycnidia mature and discharge their spores at a time. This gradual ripening of pycnidia and the subsequent discharge of the spore masses may continue for two or three months after the tube or plate is inoculated, the length of time depending upon the quantity of culture medium present and the rapidity with which it dries.

Pure cultures of this fungus on 2 per cent corn-meal agar, +0.25, are very similar to those on malt agar, except that the mycelium produced, both aerial and submerged, is much less in quantity, and no pycnidia, or only a few, are finally developed.

MORPHOLOGICAL CHARACTERS

The hyphæ of the aerial mycelium are hyalin, fairly uniform in size, ranging from 2 to 4 μ in diameter, and very sparingly branched, with septa few and distant; the submerged mycelium is dark, neutral gray to blackish slate, black when seen in mass. Often several of these hyphæ are joined into a long bundle from which individual hyphæ put off at intervals. Individual submerged hyphæ are 2 to 4 μ in diameter, sparingly branched, and distantly septate. The black submerged mycelium does not penetrate deeply into the agar, but is more or less limited to the substratum immediately beneath the aerial growth.

DISSEMINATION OF THE DISEASE

The canker, as previously stated, enters the host through wounds or dead twigs and branches. Once established in the growing inner bark, it is easy to see how other parts of the tree are infected. During every rainy or damp spell some of these spore horns which have formed on the diseased area are dissolved, and the water containing the spores runs down the tree, in this manner transmitting them to wounds or dead twigs present on the tree, and thus originating new lesions.

At present only pycnospores are known, and since these are borne in gelatinous threadlike horns, it is impossible to state definitely with our present knowledge of this fungus how it travels from tree to tree. It is probable, however, that large numbers of these spores, after once being freed from the spore horns by rains, dry and are carried by the wind to other trees. Many spores are washed into the soil at the base of the

infected trees, and the high winds common in the semiarid regions of the Southwest could easily lift into the air the spore-laden particles of dirt and carry them long distances, thereby infecting other trees. Birds and insects may also play a minor rôle in carrying the pycnospores. In the towns and cities of the Southwest where this disease is most prevalent, the only bird present to any extent is the English sparrow.

The shipment of nursery stock infected with this disease may explain its presence in many isolated places where there is no natural growth of its host to normally harbor and transmit the disease. An instance of this kind was seen at a home in the plains country in eastern New Mexico. Two species of poplar (*Populus deltoides* and *P. italica*) had been planted from two different nurseries. Sixty-five per cent of the young trees obtained from one of these nurseries were either dead or were seriously attacked by the disease within four years, and the trees from the second nursery were in good condition, except here and there a tree was being infected from the diseased material from the other nursery. In this instance the disease was apparently introduced in the nursery stock.

CONDITIONS UNDER WHICH CYTOSPORA CHRYSOSPERMA BECOMES A SERIOUS PARASITE

There are four general conditions under which this disease usually does much damage in the Southwest: (1) On trees which are growing at the outer limits of their range and are therefore in more or less unfavorable environment; (2) On trees planted in streets, lawns, and cemeteries, where they have been weakened from neglect and lack of sufficient water; (3) On trees which have been severely pruned, as in pollarding; (4) On cuttings in propagating beds, where the usual method of propagation is used.

When poplars are growing at the extreme limits of their range or are planted in the treeless regions of semiarid countries, *Cytospora chrysosperma* becomes a serious parasite. The aspen at the lower limits of its range is often attacked and the smaller trees are killed outright by this disease, while the larger trees are sometimes seriously injured. The instance given under the dissemination of this disease through nursery stock in eastern New Mexico (p. 337) where 65 per cent of the trees were killed shows how virulent this fungus can become when once established on plants growing in treeless regions. The following is another instance of the same kind from western Kansas.

Near Syracuse, in Hamilton County, many of the Carolina poplar trees (*Populus deltoides*) planted as shade trees are being killed by this disease.[1] Syracuse is located in the western portion of Kansas where trees are very scarce and where the rainfall is very light. In such a region trees are

[1] The data concerning the presence of this disease at Syracuse were kindly furnished by Prof. L. E. Melchers, of the Kansas Agricultural College Experiment Station.

considered a great luxury, and any person who succeeds in growing them always takes the greatest care not to lose them. The disease seems to be very virulent and is reported to be killing practically all of the Carolina poplars in that region. This illustrates how serious this disease becomes on species of poplar which are growing under unfavorable conditions.

The canker seems to be widely distributed in North Dakota and is causing serious damage to the poplar groves of that State, according to the following extract from a letter to the writer from State Forester Fred W. Smith:

> The poplar groves in this State are pretty largely infected with *Cytospora chrysosperma*. This disease seems to be affecting all of the poplars with the exception of the native poplars, *P. balsamifera* and *P. tremuloides* var. *candicans*. The common poplars distributed by the nurseries seem to have been all badly infected. Tens of thousands of trees have been killed in North Dakota this last year by this disease.

Poplars, when planted in dry climates on streets, lawns, and in cemeteries, are often very subject to the attacks of *Cytospora chrysosperma*, especially when the young trees are not watered regularly and abundantly. When the trees do not receive enough water, the twigs and small branches in the top gradually die. Through these dead and dying branches the fungus readily enters. This is especially true of certain species of poplar which are highly susceptible to the disease. Hundreds of shade trees in Arizona, New Mexico, and Texas have been seen by the writer which were either dying or had been seriously injured by this fungus. Most of these trees had been much neglected and undoubtedly had not had a sufficient amount of water.

In many of the towns of Arizona and New Mexico the habit of pruning large living limbs, and, in some cases, of pollarding the entire tree, is common. When large limbs are cut off in this dry climate, the exposed ends of the portion left on the tree die back from drying some 3 to 12 inches. The tree may then put out new branches below the dead portion. Later, this young growth is often killed by the gradual advance of this canker which entered at the exposed dead surface of the severed branches.

The writer has received specimens of Carolina poplar cuttings taken from propagation beds attacked by this fungus. The method usually followed in propagating trees of the genus Populus in nurseries is to take cuttings about 8 to 10 inches long and 0.25 to 0.5 inch in diameter and place them in the ground with the upper part projecting some 2 to 3 inches. Such propagation stock is often seriously damaged by *Cytospora chrysosperma*. This fungus attacks the exposed ends of the cuttings and often kills them before any shoots appear, or the cuttings may put out sprouts which are later killed by the fungus which gradually travels down the cutting into the bark below the young shoot, thereby girdling the stem and shutting off the food supply from the sprout (Pl. 28, D).

How serious this disease may be when it attacks poplar cuttings in the propagating beds is shown by the following data[1] from the Fort Hays Experiment Station nurseries situated at Hays, Kans. In 1915 two different plots containing about 10,000 Carolina poplar cuttings each were planted. On one of these plots situated on level creek-bottom land about 95 per cent of the cuttings started to grow. When the larger ones were about 10 inches tall, they began to turn yellow, quit growing, and gradually died until there was less than 1 per cent alive at the close of the season, and these were only 1 to 2 feet high. The second block of cuttings was located about 20 rods from the other block. These cuttings started nearly as well as those in block 1, but gradually died in the same manner until only about 40 per cent were alive at the end of the season. Most of the plants in this second bed grew about 3 feet high, a few were 4 to 5 feet tall, and many were only 1 to 2 feet in height. Specimens of the diseased cuttings from these two plots examined by the writer had the typical red spore horns of *Cytospora chrysosperma* which had entered at the exposed cut ends of the cuttings and had finally killed them.

The remedy for this loss in cuttings is to change the system of propagation for those species of poplar which are susceptible to the disease. The writer has seen the following method of propagating the Carolina and Lombardy poplars used with good success in the dry regions of New Mexico. The small branches or twigs which have been selected for propagating purposes, instead of being cut into 6- or 8-inch pieces, are placed entire in the bottom of a trench 2 to 4 inches deep. They are then entirely covered with sand, kept damp by frequent watering, but well drained; and in due season the dormant buds along the twigs grow and send up shoots, while an abundance of roots develop along the buried twig. Propagating stock thus handled does not present any exposed surface to be infected by the fungus.

DISTRIBUTION OF THE FUNGUS

Cytospora chrysosperma is rather widely distributed in certain sections of the United States, especially in the Southwestern States. It ranges from Texas and Kansas northward to Montana and westward to California. It has been found in nine States—Arizona, Colorado, Kansas, Montana, Nevada, New Mexico, North Dakota, South Dakota, and Texas; and also in Mexico. The fungus is widely distributed in Europe, having been reported from Germany, Austria, Switzerland, Italy, France, and Sweden.

[1] The above data were obtained through the kindness of Mr. J. W. Preston, formerly in charge of the Fort Hays Experiment Station.

DISTRIBUTION IN THE UNITED STATES

Cytospora chrysosperma has been reported from and collected[1] in the following places in the United States:

On *Populus* sp. (Alamo poplar):
El Paso, Texas, August, 1915.

On *Populus acuminata:*
Cascade, Montana, by WILLIAM LOCHRAY, August, 1913 (FP 20705).[2]
Albuquerque, May, 1916; **Chester,** November, 1916 (FP 21716); and **Deming,** New Mexico, November, 1916 (FP 21030).
Brookings, by H. F. COE, August, 1913 (FP 15799), and **Spearfish,** South Dakota, by H. F. Coe, September, 1913 (FP 15975).

On *Populus alba*:
Albuquerque, New Mexico, September, 1915, and July, 1916 (FP 19515, 19621, and 21250).
El Paso, Texas, August, 1915.

On *Populus angustifolia:*
Flagstaff, Arizona, July, 1915.
Denver, Colorado, by E. BETHEL, June, 1913 (FP 8460).
Roswell, November, 1916, and **San Mateo Mountains**, New Mexico, August, 1915 (FP 19586).

On *Populus balsamifera-suaveolens:*
Ulander, North Dakota, by B. T. GALLOWAY, September, 1916 (FP 21508).

On *Populus deltoides:*
Flagstaff, Arizona, July, 1913 (FP 19510).
Hays, by J. W. PRESTON, December, 1915 (FP 21029), and **Syracuse,** Kansas, by L. E. MELCHERS, 1915.
Golconda, Nevada, 1908 (FP 1260).
Albuquerque, August, 1915, and January, 1917 (FP 19500 and 21766); **Capitan,** by J. W. O'BYRNE, July, 1917 (FP 2937); **Deming,** December, 1915; **Endee,** by A. S. REEVES, April, 1915 (FP 19503), and May, 1915; and **Socorro,** New Mexico, July, 1915 (FP 19587).
El Paso, August, 1915; and **San Marcos,** Texas, November, 1915 (FP 19726).

On *Populus italica:*
Flagstaff, Arizona, July, 1915 (FP 19448).
Albuquerque, throughout the entire year 1916; by P. W. SEAY, December, 1916 (FP 21762 and 21765); **Isleta**, New Mexico, November, 1916 (FP 21588).
El Paso, Texas, August, 1915.

On *Populus macdougali:*
Phoenix, December, 1915; **Tucson,** December, 1915; and **Yuma,** Arizona, December, 1915 (FP 21026); **Deming,** New Mexico, December, 1915 (FP 21031).

On *Populus sargentii:*
Denver, Colorado, by E. BETHEL, June, 1913 (FP 8431).

On *Populus tremuloides:*
Flagstaff, Arizona, July, 1915 (FP 19447).
Cienega Ranger Station, 1914 (FP 19622); **Cloudcroft,** August, 1915 (FP 19623); and **Tejano Experiment Station,** New Mexico, by P. W. SEAY, December, 1916 (FP 21718).

[1] All of the collections cited were made by the writer unless otherwise stated.
[2] "FP"=Forest Pathology Investigations.

On *Populus wislizeni:*
Flagstaff, Arizona, July, 1916 (FP 21262).
Albuquerque, November, 1915 (FP 19919); by R. M. HARSCH, September, 1916 (FP 21605); and **Domingo,** New Mexico, by R. M. HARSCH, August, 1915.
El Paso, August, 1915; and **Pecos,** Texas, November, 1916 (FP 21717).
On *Salix amygaloides:*
Denver, Colorado, by E. BETHEL, June, 1913 (FP 8432).
On *Salix babylonica:*
Albuquerque, New Mexico, September and August, 1915 (FP 19493).
On *Salix wrightii:*
Yuma, Arizona, December, 1915 (FP 21764).

DISTRIBUTION IN MEXICO

On *Populus* sp. (Alamo poplar), **Juarez,** 1912.
On *Populus italica,* **Juarez,** 1912.
On *Populus wislizeni,* **Juarez,** 1912.

From the foregoing data it will be noted that 14 species of trees are attacked by *Cytospora chrysosperma,* as follows: *Populus acuminata, P. alba, P. angustifolia, P. balsamifera-suaveolens, P. deltoides, P. italica, P. macdougali, P. sargentii, P. tremuloides, P. wislizeni, Populus* sp. (Alamo poplar), *Salix amygdaloides, S. babylonica,* and *S. wrightii.*

CONTROL OF THE DISEASE

Certain species of poplars have been found to be more susceptible to this disease than others. Therefore, only those species of poplars which are most resistant to the disease should be selected for planting in regions where this disease is common. The Carolina poplar and the silver-leaf poplar are highly susceptible to this disease under the conditions obtaining in a dry climate like that of the Southwest. When these two species of trees are about 12 to 14 inches in diameter at the ground, they usually show, in the crown, an increasing number of large, dead branches which have been killed by *Cytospora chrysosperma.* In fact, the writer has never examined a large tree of either of these two species in Arizona or New Mexico that has not been seriously injured or finally killed by the disease. This is particularly true in the vicinity of Albuquerque, N. Mex. The Carolina poplar is also attacked in this western country by two insect parasites which weaken the trees and make them unsightly and more easily infected by this disease. One is the cottony scale (*Pulvinaria* sp.), which usually seriously deforms the trees, and the other insect is the poplar borer (*Saperda* sp.). This species of poplar has, therefore, three serious enemies with which to contend in this arid country and should not be planted as an ornamental or shade tree.

The native valley cottonwood (*Populus wislizeni*) is highly resistant to the disease caused by *Cytospora chrysosperma* when given any kind of care and attention and should be selected for planting in those portions of the Southwest within its range. On account of the large amount

of cotton produced by the pistillate trees of the valley cottonwood, only the staminate or non-cotton-bearing trees should be planted.

The Lombardy poplar is also resistant to this disease, but not as much so as the valley cottonwood.

Cytospora chrysosperma is not an active vigorous parasite on the well-cared-for, more resistant species of poplar. It therefore follows that in controlling this disease the trees should be given plenty of water, and care should be taken not to injure the bark of the tree in any way; particularly should the cutting off of large branches be avoided. In general, the most resistant species for planting should be selected, the trees should be given a sufficient amount of water, and should be protected against injury from lawn mowers, horses, etc. Trees thus selected and taken care of will be practically immune to the disease.

Since this disease is known to be a serious parasite in nurseries and propagating beds and to be distributed by means of such diseased stock, it is of the utmost importance that all nurseries which supply poplar stock to the dry regions of the western United States should be inspected for this and other serious diseases of poplars like *Dothichiza populea*.[1] All suspicious as well as plainly diseased stock should be destroyed by burning. General precautions of this nature, if taken, will do much to control the introduction of this disease into new territories.

If a tree already has this disease, it may often be saved by cutting off the infected branches at least 12 inches below where any signs of the disease can be detected. The ends of the branch should be painted with creosote or coal tar. If the tree is small and has a large canker on the main stem involving more than one-third of the circumference of the tree at that point, it would be best either to plant another tree or to cut the tree back to the ground and let one of the most vigorous suckers or sprouts grow. It would be impossible to cut out all of the diseased tissue on the trunk of a small tree without practically girdling it.

If the tree is 12 inches or more in diameter where infected, and the lesion is small, the disease may possibly be eradicated by cutting out all of the diseased bark and the discolored sapwood. A layer of sound bark 2 inches wide should then be cut from around the diseased area with a thoroughly sterilized knife. All of the surface exposed by the pruning operation should be painted with a strong solution of shellac or coal tar to prevent reinfection.

SUMMARY

(1) A serious canker of poplars and willows is prevalent throughout the semiarid regions of the southwestern United States.

(2) This disease is caused by *Cytospora chrysosperma*.

[1] HEDGCOCK, G. G., and HUNT, N. R. DOTHICHIZA POPULEA IN THE UNITED STATES. *In* Mycologia, v. 8, no. 6, 300–308, pl. 194–195, 1916. Literature cited, p. 308.

(3) Pure cultures of this fungus were isolated from the diseased areas, and the typical lesions of the disease were produced by inoculating healthy poplar plants with pure cultures of the fungus. The fungus was reisolated from the cankers produced by the inoculations.

(4) Pure cultures of *C. chrysosperma* on malt and corn-meal agars were made, and the cultural characters of the fungus determined.

(5) The fungus enters the host through wounds and dead branches.

(6) *C. chrysosperma* is a serious parasite on poplars in the Southwest under the following conditions: (a) On trees which are growing at the outer limits of their range and are therefore in a more or less unfavorable environment; (b) on trees planted in streets, lawns, and cemeteries where they have been weakened from neglect and lack of sufficient water; (c) on trees which have been severely pruned, as in pollarding; (d) on cuttings in propagating beds where the usual method of propagation is used.

(7) The fungus *C. chrysosperma* occurs in nine States and on 14 different species of trees. It is also found in Mexico and in Europe.

(8) The best method of controlling this disease is as follows: (a) The most resistant species should be selected; the trees should be given an abundance of water, and should be protected against mechanical injuries; (b) a strict supervision should be established over all nurseries handling poplar stock intended for distribution in the semiarid regions of the western United States; (c) all nursery stock which shows the slightest indication of the disease should be destroyed.

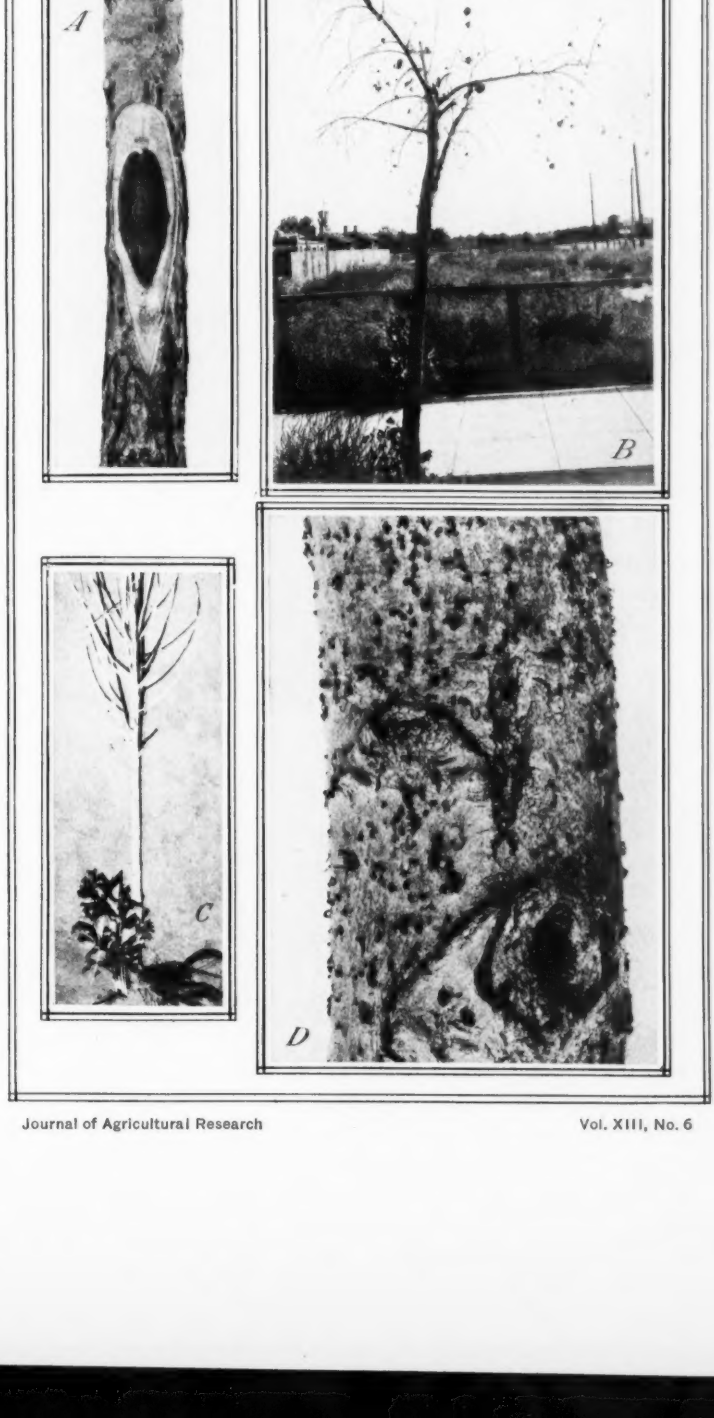
PLATE 27

A.—A small canker caused by *Cytospora chrysosperma* on the trunk of a tree of *Populus italica*, with the bark cut from around canker.

B.—A tree of *Populus wislizeni* on the streets of Albuquerque, N. Mex., dying from the attacks of *C. chrysosperma*.

C.—Main stem of a young tree of *Populus italica* killed by *C. chrysosperma*, showing young sprouts at the base of the tree.

D.—A branch of *Populus wislizeni* attacked by *C. chrysosperma*, showing the spore horns of the fungus.


A
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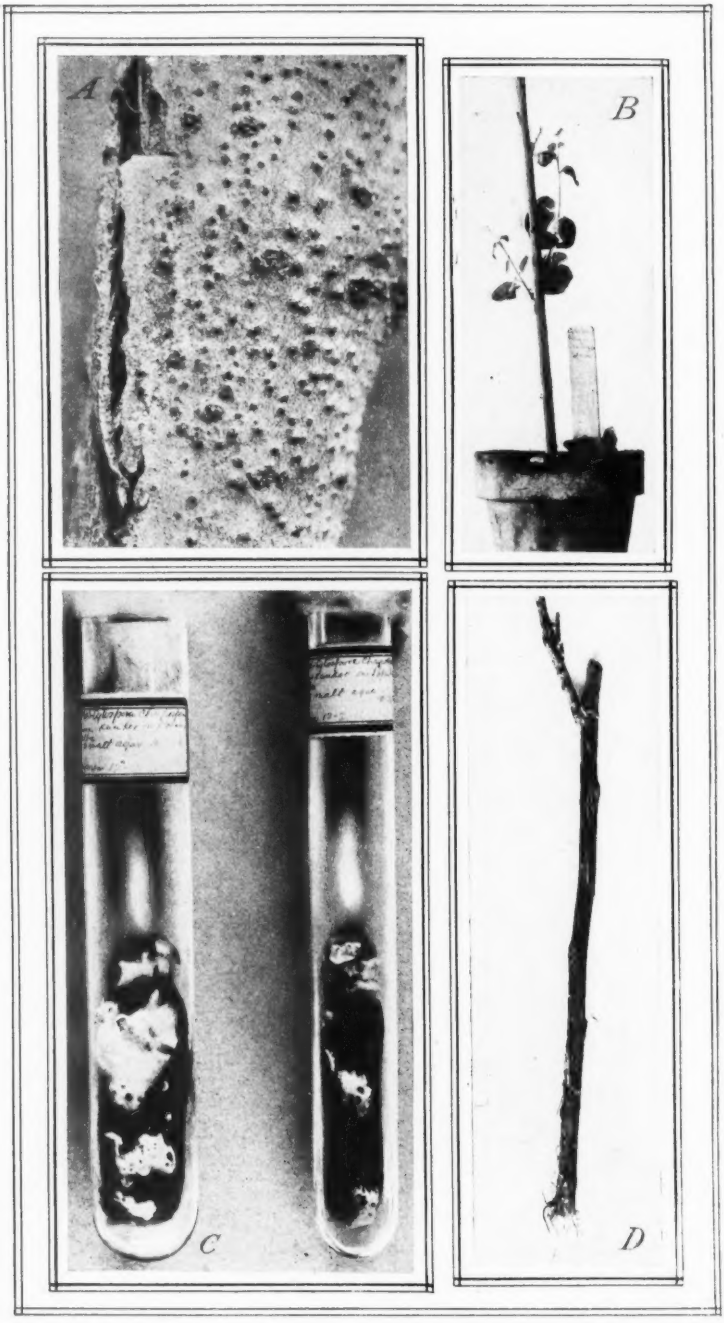
A
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PLATE 28

A.—Pycnidia of *Cytospora chrysosperma* on *Populus alba* after the spore horns have been washed away by rains.

B.—A young plant of *Populus italica*, showing the upper portion of the stem killed by inoculation with *C. chrysosperma*. Sprouts are putting out below the point of inoculation.

C.—Two tubes of pure cultures of *C. chrysosperma* on malt agar, showing pycnidia and spore droplets.

D.—A propagation cutting of *Populus deltoides* from Hays, Kans., killed by *C. chrysosperma*.